Glucose-evoked transforming growth factor reduces connexin and purinergic signalling in proximal tubule-derived epithelial cells.

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Aim: In diabetic nephropathy, glucose-evoked TGF-beta1 induced tubular injury reduces E-cadherin mediated cell-to-cell adhesion. Co-localized with E-cadherin at the sites of cell-cell contact, connexins (Cx) form either gap junctions or hemi-channels through which ATP is secreted. This study determines how TGF-beta evoked changes in Cx-expression may be linked to alterations in P2-purinergic receptor expression/function.

Methods: The effect of TGF-beta1 on clonal HK2 membrane integrity was assessed by MTT and a lactate-dehydrogenase (LDH) assay. Western blot analysis confirmed changes in expression of the connexins Cx26, Cx40 and Cx43 and the purinergic receptors P2Y1, P2Y2 and P2Y6 +/- TGF-β1. Efficacy profiles for various purinergic receptor agonists were determined by calcium microflourimetry.

Results: At 48hrs TGF-beta1 decreased expression of Cx26 as compared to control to 44.0±7.5%, 38.9±11.0% and 25.8±6.3% at 2, 4 and 10ng/mL and Cx43 to 61.6±7.8%, 53.4±11.4% and 33.1±11.2% (n=3; $p<0.01$). The effects were not dependent on changes in membrane integrity as assessed by an LDH assay. Expression of Cx40 was unaffected by the pro-fibrotic cytokine. TGF-beta1 (48hrs) decreased P2Y1 protein expression by 75.3±3.9% ($p<0.05$), 58.0±4.1% ($p<0.01$) and 48.2±8.7% ($p<0.001$) at 2, 4 and 10ng/mL (n=3) and P2Y6 expression by 40.4±9.9% at 10ng/mL ($p<0.05$, n=3). The protein expression of P2Y2 was unaffected by TGF-beta1. P2-purinergic receptor agonists evoked a concentration-dependent (0.1-100μM) increase in cyctosolic calcium, with an efficacy profile of ATP≥UTP>>ADP>>AMP=Adenosine.

Conclusions: TGF-beta1 reduced Cx26 and Cx43 expression in HK2-cells, an effect mirrored by a loss in P2Y1 and P2Y6 expression. These data suggest that TGF-beta may exacerbate hemi-channel dependent intercellular communication in proximal tubule-derived epithelial cells.

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